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Exogenous addition of nitrate nitrogen regulates the uptake and translocation of lead (Pb) by *Iris lacteal* Pall. var. *chinensis* (Fisch.) Koidz.

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Abstract: Since Pb is a non-biodegradable inorganic pollutant and a non-essential metal, its long-term presence in soil poses a great threat to the environment. *Iris lactea* Pall. var. *chinensis* (Fisch.) Koidz., a perennial dense bush herb with high resistance of Pb and wide adaptability, was used in pot experiments to study the effects of exogenous nitrate N (NO_3^--N) on the absorption and transportation of Pb and plant growth under different Pb concentrations. Then, the mechanism of NO_3^--N affecting Pb and nutrient uptake and transport was explored. The concentration of Pb in the experiment ranged from 0 to 1600 mg/kg, and the added concentration of NO_3^--N was 0.0–0.3 g/kg. The results showed that *I. lactea* was highly tolerant to Pb, and the shoot fraction was more sensitive to varied Pb concentrations in the soil than the root fraction. This protective function became more pronounced under the condition of raised Pb concentration in the soil. When the concentration of Pb in the soil reached 800 mg/kg, the highest Pb content of *I. lactea* was found under the condition of 0.1 g/kg of NO_3^--N addition. When Pb concentration in the soil increased to 1600 mg/kg, the increase in NO_3^--N addition promoted Pb uptake by the root. To ensure the well growth of *I. lactea* and the effect of remediation of Pb-contaminated soil, the recommended concentration of NO_3^--N in the soil is 0.1 g/kg. This result provides a theoretical basis for exogenous N regulation of phytoremediation of Pb-contaminated soil.

Keywords: *Iris lactea*; nitrate nitrogen; plant nutrient; lead accumulation; absorb; transport

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1 Introduction

Lead (Pb) pollutants can enter soil environment through various ways, causing severe soil pollution (Sinha et al., 2006). Over the past 50 a, approximately 7.83×10^5 t of Pb has been discharged into the global environment, resulting in varying degrees of Pb pollution in the soil around the world (Duzgoren-Aydin, 2007). Since Pb is a non-biodegradable inorganic pollutant as well as a non-essential metal, its long-term persistence in soil is a great threat to the environment (Amari et al., 2017; Gerhardt et al., 2017). Excessive Pb enters the environment and participates in the water-soil-biological system cycle. It accumulates in the roots, stems, leaves, and seeds of plants through the absorption of plants, and endangers the health of animals and human beings through the enrichment of food chain (Salazar et al., 2016; He et al., 2019). Pb is highly toxic and

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hazardous to humans as well as plants even at low concentrations. The excessive amount of Pb in soil may cause stress-induced changes in plants including growth reduction, decreased biomass, leaf chlorosis, and other physiological and biochemical changes (Chandana et al., 2019).

Pollution remediation methods of heavy metal in soil mainly include physical methods (e.g., landfilling and replacement with uncontaminated soil), and chemical methods (e.g., chemical fixation, leaching, and extraction) (Park and Son, 2017; Liu et al., 2018). The above methods are inefficient and costly to operate, and may also cause damage to the original soil biological environment. Due to the decreased mobility of Pb in soil, tolerant plants may concentrate Pb in their roots. Studies have shown that the ability to translocate Pb into the aboveground parts may be very low (Salazar and Pignata, 2014). In this context, testing of metal tolerant plant species whether capable of accumulating Pb from soil into their roots or translocating into shoots becomes highly significant. In recent years, *in situ* remediation of contaminated soils using heavy metal hyperaccumulator plants has been accepted as very promising method. The phytoremediation method is characterized by the high efficiency, no secondary pollution, long-lasting effect, low cost, and easy application (Chaney et al., 2005; Han et al., 2008). For example, *Helichrysum microphyllum* subsp. *tyrrhenicum* is an endemic plant species in Sardinia and Corsica, Europe, with good tolerance to heavy metals such as zinc (Zn), Pb, and cadmium (Cd). It was used as a pioneer species for mine soil remediation by Boi et al. (2021).

Several exogenous substances can affect the uptake and transport of heavy metals by plants. The most studied exogenous substances are chelating agents and fertilizers (Vassil et al., 1998; Shen et al., 2002). Nitrogen (N) fertilizer mainly affects the activity of heavy metals through the acidification and alkalization effects of NH_4^+-N and NO_3^--N in the rhizosphere (Zeng et al., 2020). When NH_4^+-N is absorbed, the plant secretes H^+, causing acidification around the rhizosphere; while absorbing NO_3^--N, the plant secretes OH^-, causing alkalinization of the rhizosphere (Wallace, 1979). Pb contents in the roots and shoots of maize treated with NH_4^+-N were significantly higher than those treated with NO_3^--N, with an increase of about 20%. And the application of NH_4^+-N fertilizer can increase the content of Pb in plant tissues (Lou et al., 2005). Some studies have pointed out that NO_3^--N can promote the uptake and transport of heavy metals by plants. Nitrate fertilization has been shown to increase Zn hyperaccumulation in *Nocceaea caerulescens* (J. Presl & C. Presl) F.K. Mey (Schwartz et al., 2003; Monsant et al., 2008; Xie et al., 2009).

I. lactea is a perennial herb that is distributed in the northern temperate regions of Asia, Europe, and North America. It also has a wide distribution in northwestern, northeastern, and northern China. Simultaneously, being a long-term growth and ornamental plant, *I. lactea* has strong tolerance and uptake capacity for Pb (Bai et al., 2008; Ayyasamy et al., 2009). Suzhen et al. (2018) found that the content of Pb in the shoots (leaf and stem) of *I. lactea* was 983 mg/kg, and the transport coefficient was greater than 1, indicating it has the potential to accumulate high amounts of Pb in its tissues (Yuan et al., 2018). Han et al. (2008) speculated that *I. lactea* can sacrifice part of its cells to adsorb Pb, thus reducing the toxicity of Pb and ensuring the normal growth and development of the plant. The results of hydroponic tradition showed that Pb contents in the aerial part and root of *I. lactea* were 1109 and 2408 mg/kg, respectively, exceeding the standard for hyperaccumulators (1000 mg/kg). Thus, the application of *I. lactea* to remediate Pb-contaminated soils is feasible (Han et al., 2013). However, the remediation capacity of *I. lactea* under exogenous addition of fertilizer based on soil culture has apparently not been investigated, while previous studies on the enrichment of *I. lactea* for Pb were hydroponics. Under different culture conditions, Pb accumulation and translocation to the shoots, accumulation mode, and detoxification mechanism of plants were relatively different. Accumulation of Pb in *I. lactea* under soil cultivation was significantly reduced compared with that under solution culture condition, especially in the shoots. The reason for this may be related to the low water solubility of Pb and the less available Pb in soil that can be uptaken by plants (Zhuang et al., 2000). In conclusion, NO_3^--N can significantly promote the uptake of heavy metals by plants, but the remediation potential of *I. lactea* in Pb-contaminated soils remains unclear. In the present study,

the effects of exogenous NO_3^--N on the absorption and transportation of Pb, nutrient accumulation, and growth of *I. lactea* under different Pb concentrations were studied. The result might provide sustainable management and remediation for Pb-contaminated soils.

2 Materials and methods

2.1 Plants and soil sampling

I. lactea seeds and soil samples were obtained from the Yanqing Experimental Base of Beijing Oasis Technology Co., Ltd., China. Soil samples were sieved to a particle size of <2 mm to exclude the coarse and large debris, and were air-dried (22°C) for 1 week. Soil properties are shown in Table 1.

Table 1 Physical-chemical properties of the soil

pH	EC (μS/cm)	CEC (cmol/kg)	SOM (g/kg)	Total N (g/kg)	Available P (mg/kg)	Available K (mg/kg)	Total Pb (mg/kg)
8.22	198.00	13.81	12.67	0.59	13.06	146.32	17.56

Note: EC, electric conductivity; CEC, cation exchange capacity; SOM, soil organic matter; N, nitrogen; P, phosphorus; K, potassium; Pb, lead.

2.2 Pot experiment

Plants were grown in the plant cultivation room (25°C (±1°C)/12 h light, 22°C (±1°C)/12 h dark, and 63% relative humidity) of the Department of Soil and Water Sciences, School of Land Science and Technology, China Agricultural University. After disinfection with 3% H_2O_2 and repeated washing with deionized water, *I. lactea* seeds were mixed with river sand sterilized at 105°C and refrigerated at 4°C. After two months, seeds were disinfected with 0.5% NaClO for 20 min. After washing with tap water, seeds were germinated in a continuous dark incubator at 25°C using the sand bed tissue culture method. After germination, seeds were irrigated with 1/4 nutrient solution. The formula of nutrient solution is shown in Table S1. The nutrient solution gradually increased from 1/2 concentration to total nutrient solution. During the culture period, pH of nutrient solution was adjusted to about 6.0.

Pb was added in the form of $Pb(CH_3COO)_2 \cdot 3H_2O$ at concentrations of 0 (Pb_0), 800 (Pb_1), and 1600 (Pb_2) mg/kg. N fertilizer was added in the form of $NaNO_3$ in the following concentrations: 0.0 (N_0), 0.1 (N_1), 0.2 (N_2), and 0.3 g/kg (N_3) (Table 2). A total of 12 treatments were set for the whole experiment (Table 2), and each treatment was repeated three times. Phosphate and potash fertilizer were added in the form of $KH_2(PO_4)_3$, and P_2O_5 and K_2O concentrations were 0.20 and 0.25 g/kg, respectively. The soil was mixed with Pb and base fertilizer, and loaded into a plastic pot (12.0 cm in diameter and 13.5 cm in height), to a bulk density of 1.4 g/cm^3. After culturing in

Table 2 Experimental treatment

Treatment	Pb (mg/kg)	NO_3^--N (g/kg)
CK (control)	0	0.0
Pb_0N_1	0	0.1
Pb_0N_2	0	0.2
Pb_0N_3	0	0.3
Pb_1N_0	800	0.0
Pb_1N_1	800	0.1
Pb_1N_2	800	0.2
Pb_1N_3	800	0.3
Pb_2N_0	1600	0.0
Pb_2N_1	1600	0.1
Pb_2N_2	1600	0.2
Pb_2N_3	1600	0.3

the greenhouse for 2 weeks, seedlings with the same growth trend and height of 5 cm were transplanted to a pot with six seedlings for 8 weeks and watered appropriately to maintain 70% of the field capacity.

2.3 Sample collection

After 45 d of treatment, plants were harvested from roots and shoots, and potted soil samples were collected at the same time. Plant samples were treated at 105°C for 15 min, dried at 75°C to constant weight, and weighed. Samples were ground and sieved to determine the contents of nutrient elements and Pb. Soil samples were ground and sieved after air drying to determine soil pH, soil nutrient, and soil Pb contents.

2.4 Measurements

The forms of Pb in the soil were determined using the modified BCR (Bureau of Reference, European Community) three-step sequential extraction procedure (Rauret et al., 1999; Bao, 2000) (Table 3). Pb content of plants was determined by HNO_3-$HClO_4$ digestion and inductively coupled plasma atomic emission spectrometry (Perkin Elmer, Waltham, USA). Total N, phosphorus (P), and potassium (K) contents of plants were determined using the Kjeldahl method. P was determined by the vanadium molybdenum yellow colorimetry method. K was determined by the flame photometer method after dry plant samples were digested by H_2SO_4-H_2O_2 (Stanisław et al., 2017). The biomass was determined by drying the plants and weighing them with a 1/10,000 balance. Transfer coefficient of Pb is expressed as:

$$\text{Transport coefficient}=\text{aboveground Pb content}/\text{belowground Pb content}. \quad (1)$$

Table 3 BCR (Bureau of Reference, European Community) three-step sequential extraction procedure

Step	Extraction form	Chemical reagents and extraction condition
1	Acid soluble	Taking 1 g of air dried soil into a 100-mL centrifuge tube, adding 40 mL of 0.11 mol/L HOAc, shaking at 22°C (±5°C) for 16 h, centrifuging for 20 min at 3000 r/min, and then transferring the supernatant to a polyethylene bottle.
2	Reducible	Adding 40 mL of 0.5 mol/L $NH_2OH·HCl$ (pH 1.5) solution containing 25 mL HNO_3 to the solid residue in step 1, shaking at 22°C (±5°C) for 16 h, centrifuging for 20 min at 3000 r/min, and then transferring the supernatant to a polyethylene bottle.
3	Oxidizable	Adding 10 mL of 8.8 mol/L H_2O_2 (pH 2–3) to the solid residue in step 2, keeping at room temperature for 1 h, and water bathing at 85°C (±2°C) for 1 h. When heated to a volume less than 3 mL, adding another 10 mL of H_2O_2, then water bathing at 85°C (±2°C) for 1 h. When heating to a volume less than 1 mL, adding 50 mL of 1 mol/L NH_4OAc (pH 2), centrifuging for 20 min at 3000 r/min, and then transferring the supernatant to a polyethylene bottle.
4	Residual	Differential subtraction method.

2.5 Statistical analysis

SPSS software package v.24.0 was used for data processing, and Origin Pro v.8.5 was used for visualizing the results. The variance method was adopted to analyze the significance of the relation between different amounts of NO_3^--N and the adsorption of Pb by *I. lactea* (*F* test with a significance threshold of 0.05). Difference between treatments was tested by the LSD (least significant difference) method with a significance level of 0.05.

3 Results

3.1 Biomass

Under Pb_0 treatment, shoot biomass of *I. lactea* showed an overall decreasing trend with increasing NO_3^--N concentration. Shoot biomass of *I. lactea* was not significantly different among CK, N_2, and N_3 treatments, but significantly lower than N_1 treatment (Fig. 1). Shoot biomass of *I. lactea* under Pb_1 treatment was slightly higher than those under Pb_0 and Pb_2 treatments, and root biomass decreased with the increase of NO_3^--N concentration. Shoot biomass of *I. lactea* decreased significantly under Pb_2 treatment, and root biomass had no significant difference at N_0

and N_1 concentrations, but was substantially higher than those at N_2 and N_3 concentrations. Root/shoot ratio of *I. lactea* increased with the increase of NO_3^--N concentration under Pb_0 and Pb_2 treatments, but decreased first and then slowly increased under Pb_1 treatment (Table S1).

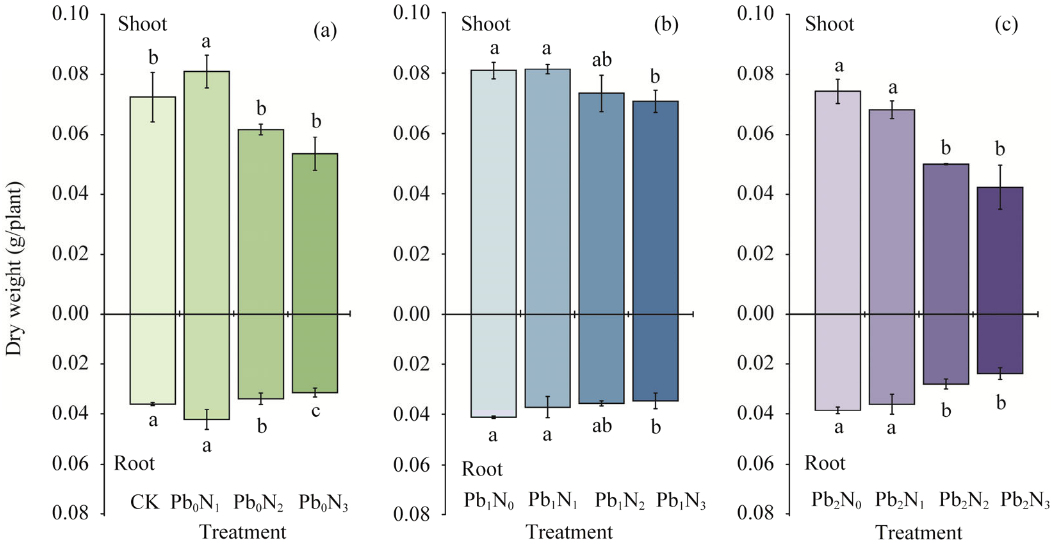

Fig. 1 Biomass of *Iris lactea* in lead (Pb)-contaminated soil with exogenous nitrate nitrogen (NO_3^--N). CK, 0.0 g/kg Pb and N; N_0, 0.0 g/kg; N_1, 0.1 g/kg, N_2, 0.2 g/kg; N_3, 0.3 g/kg; Pb_0, 0 mg/kg; Pb_1, 800 mg/kg; Pb_2, 1600 mg/kg. (a), Pb_0 treatment; (b), Pb_1 treatment; (c), Pb_2 treatment. The detailed treatment of Pb and N is shown in Table 2. Different lowercase letters within the same Pb treatment indicate significant differences among different N treatments in root or shoot at P<0.05 level according to Tukey's test. Bars are standard errors.

3.2 Effect of exogenous NO_3^--N on soil pH

Soil pH generally showed a decreasing trend with increasing exogenous NO_3^--N concentration under different Pb treatments (Fig. 2). However, there were variations in pH in the soils of different Pb treatments. Under Pb_0 treatment, the addition of NO_3^--N at all concentrations reduced soil pH to the same extent. Under Pb_1 treatment, exogenous NO_3^--N at N_2 and N_3 concentrations reduced soil pH to the same extent. While under Pb_2 treatment, NO_3^--N at N_1 concentration did not significantly affect soil pH, while NO_3^--N at N_2 and N_3 concentrations similarly reduced soil pH.

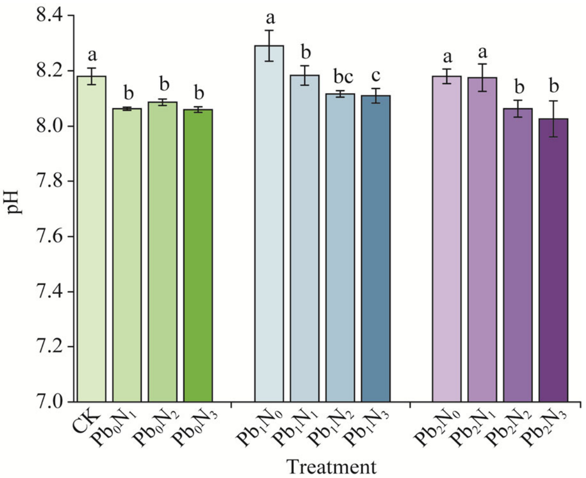

Fig. 2 pH value varied in Pb-contaminated soil with exogenous nitrate nitrogen (NO_3^--N). CK, 0.0 g/kg Pb and N; N_0, 0.0 mg/kg; N_1, 0.1 g/kg, N_2, 0.2 g/kg; N_3, 0.3 g/kg; Pb_0, 0 mg/kg; Pb_1, 800 mg/kg; Pb_2, 1600 mg/kg. The detailed treatment is shown in Table 2. Different lowercase letters within the same Pb treatment denote significant differences among different N treatments at P<0.05 level according to Tukey's test. Bars are standard errors.

3.3 Uptake and transport of N by *I. lactea*

Addition of exogenous NO_3^--N had no significant effect on N content in the root of *I. lactea* in the soil without Pb contamination (Table S2). Under Pb_0 treatment, with the increase of NO_3^--N concentration, shoot N content of *I. lactea* showed an increasing trend and then decreasing trend after reaching the maximum value at N_1 concentration. Shoot N accumulation at this time was 1.94 mg/plant, which increased by 29.33% in comparison with the treatment without NO_3^--N (Table S3). Under Pb_1N_2 treatment, the highest N accumulation in the shoot reached 1.88 mg/plant, while N accumulation in the remaining treatments was ranked as Pb_1N_1>Pb_1N_0>Pb_1N_3. Under Pb_2 treatment, addition of exogenous NO_3^--N had positive significant effect on the shoot and root N contents of *I. lactea*. Under Pb_2N_0 treatment, *I. lactea* accumulated the highest shoot N, reaching 1.16 mg/plant, which was significantly higher than other treatments. Shoot and root N contents of each NO_3^--N treatment were in the order of Pb_2N_0>Pb_2N_1>Pb_2N_2>Pb_2N_3, which significantly inhibited the translocation of N to the shoot with the increase in NO_3^--N concentration (Table S4). N uptake in the root of *I. lactea* did not differ extensively with the increase in NO_3^--N concentration.

3.4 Chemical form distribution of Pb in the soil

In each treatment, the forms of Pb mainly exist in reducible state and residue state, accounting for more than 40% and 30% of the total Pb, respectively (Fig. 3). Order of the proportion of different forms of Pb from high to low is reducible>residual>acid soluble>oxidizable. With the increase in exogenous Pb, proportion of acid soluble Pb increased, while proportion of reducible and oxidizable Pb decreased, and residual Pb did not show an obvious change trend. Under Pb_1 treatment, addition of exogenous NO_3^--N had no significant effect on the proportions of acid soluble, reducible, and residual Pb. However, proportion of oxidizable Pb significantly increased under Pb_1N_2 treatment.

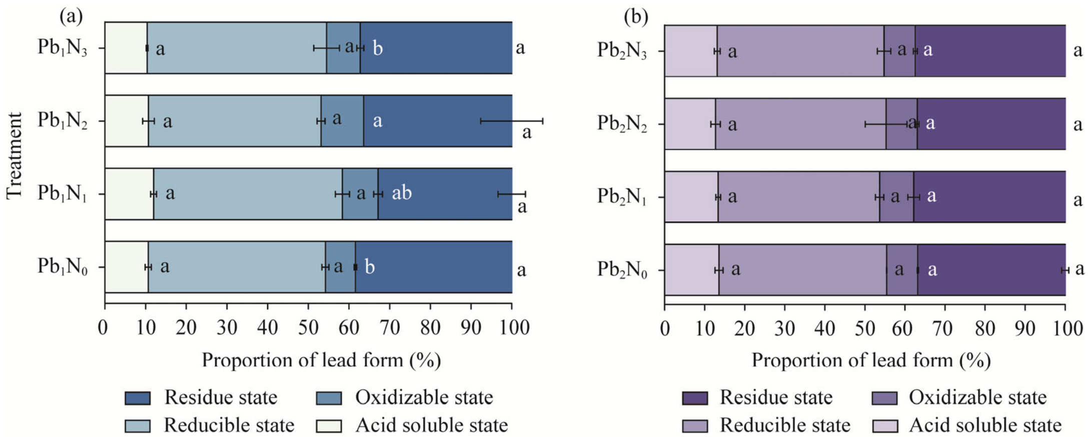

Fig. 3 Transformation of lead (Pb) and proportions in different forms with exogenous nitrate nitrogen (NO_3^--N). N_0, 0.0 mg/kg; N_1, 0.1 g/kg, N_2, 0.2 g/kg; N_3, 0.3 g/kg; Pb_1, 800 mg/kg; Pb_2, 1600 mg/kg. (a), Pb_1 treatment; (b), Pb_2 treatment. The detailed treatment is shown in Table 2. Different lowercase letters within the same Pb treatment denote significant differences among different forms of Pb at P<0.05 level according to Tukey's test. Bars are standard errors.

3.5 Absorption and transport of Pb by *I. lactea*

Under the treatments of Pb_1 and Pb_2, root Pb concentration of *I. lactea* reached up to 1744.09 and 3893.24 mg/kg, respectively, while shoot Pb concentration reached up to 111.92 and 284.30 mg/kg, respectively. Uptake and accumulation of Pb in *I. lactea* were mainly concentrated in the root, and its root Pb content was 9.6–22.1 times higher than that of Pb content in the shoot (Fig. 4).

Under Pb_1 treatment, with the increase in NO_3^--N concentration, Pb concentration in the root of *I. lactea* increased first and then decreased (Fig. 5a). Under Pb_1N_1 treatment, Pb concentration

reached the maximum, with 1744.09 mg/kg and the highest accumulation of 0.06 mg/plant, which was drastically higher than other treatments. Transport coefficients of Pb_1N_0>Pb_1N_2>Pb_1N_3>Pb_1N_1 in descending order were all less than 0.1 (Table S5).

Under Pb_2 treatment, addition of exogenous NO_3^--N promoted the absorption of Pb in the root of *I. lactea* (Fig. 5b). Addition of exogenous NO_3^--N had no significant effect on Pb concentration in the shoot of *I. lactea*. But with the increase in NO_3^--N concentration, transport coefficient decreased linearly, forming a descending order of Pb_2N_0>Pb_2N_1>Pb_2N_2>Pb_2N_3.

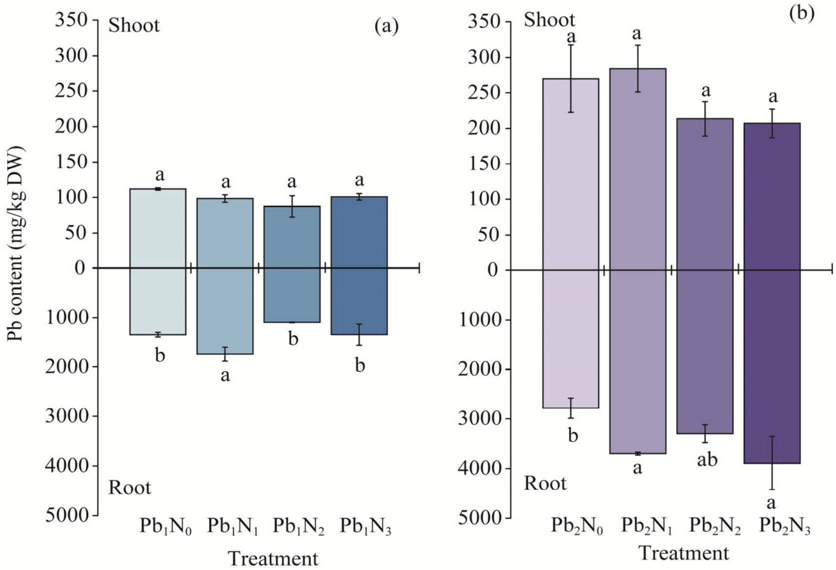

Fig. 4 Lead (Pb) content of *Iris lactea* in Pb-contaminated soil with exogenous nitrate nitrogen (NO_3^--N). N_0, 0.0 mg/kg; N_1, 0.1 g/kg, N_2, 0.2 g/kg; N_3, 0.3 g/kg; Pb_1, 800 mg/kg; Pb_2, 1600 mg/kg. (a), Pb_1 treatment; (b), Pb_2 treatment. The detailed treatment is shown in Table 2. Different lowercase letters within the same Pb treatment denote significant differences among different N treatments at P<0.05 level according to Tukey's test. Bars are standard errors.

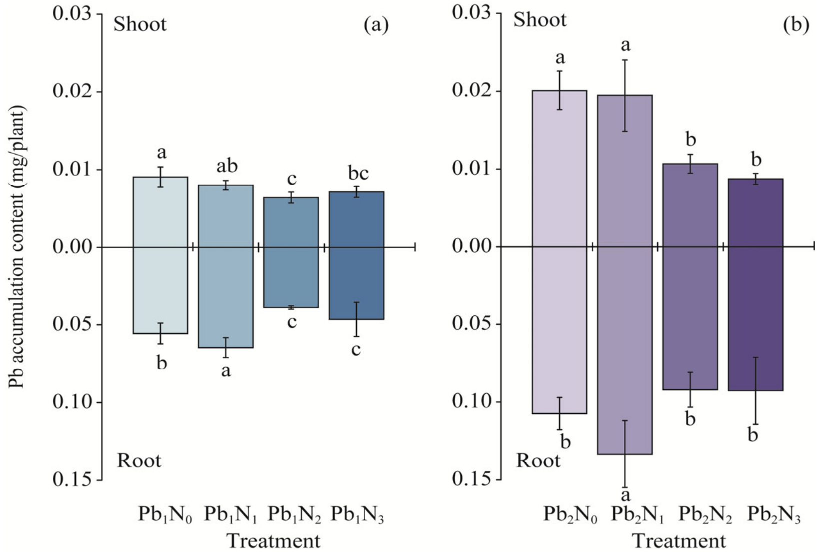

Fig. 5 Lead (Pb) accumulation content of *Iris lactea* in Pb-contaminated soil with exogenous nitrate nitrogen (NO_3^--N). N_0, 0.0 mg/kg; N_1, 0.1 g/kg, N_2, 0.2 g/kg; N_3, 0.3 g/kg; Pb_1, 800 mg/kg; Pb_2, 1600 mg/kg. (a), Pb_1 treatment; (b) Pb_2 treatment. The detailed treatment is shown in Table 2. Different lowercase letters within the same Pb treatment denote significant differences among different N treatments at P<0.05 level according to Tukey's test. Bars are standard errors.

4 Discussion

4.1 Variation of nitrogen on soil pH

The selection of appropriate N fertilizer forms and fertilization methods can be used as a strategy to control the absorption of heavy metals by crops and improve the safety of agricultural products (Hamlin and Barker, 2006). The effects of chemical N fertilizer on soil physical-chemical properties are mainly manifested via the effect on soil acidity. The reasons for change in soil pH can be attributed to two aspects. Firstly, pH change is caused by the transformation and migration of N form. When NH_4^+-N is applied to the soil, soil pH can be reduced significantly over a relatively short period through nitrification, and then NO_3^--N produced in the nitrification process or applied in the form of fertilizer can take away the base ions through leaching, further acidifying the soil (Williams et al., 1987). Secondly, change in rhizosphere pH caused by plant absorption of different forms of N and secretion of H^+ or OH^-. When different forms of N fertilizer are applied to plants, the plant rhizosphere environment exhibits distinct changes, with the root system secreting different ions after NH_4^+-N and NO_3^--N uptake. Following NH_4^+-N uptake, a root system will secrete H^+, which causes acidification around the rhizosphere, while following NO_3^--N uptake, the root system secretes OH^-, resulting in rhizosphere alkalization (Wallace et al., 1979). Different forms of N fertilizer have different effects on soil acidification and rhizosphere environment, thus resulting in the variation of adsorption of heavy metals in the soils (Lou et al., 2005).

4.2 Effect of nitrate nitrogen addition on lead content

Previous studies have reported different forms of Pb in the soil (Wu et al., 2013). Rosik-Dulewska and Karwaczyńska (2004) found that Pb enters the soil mainly in the form of residue; conversely, Lee et al. (2015) showed that Pb was predominantly found in carbonate and reducible fractions in soil. In this study, we found that Pb mainly exists in a reducible state after entering the soil but that residual state also accounts for a large proportion. Pb content in the reducible and residual states accounts for >78% of the total amount. The reason for the differences among these studies may be related to the soil used. There is a very significant and positive correlation between Pb contents of root and shoot of *I. lactea* and Pb contents of various forms in the soil, among which the correlation with the content of acid soluble Pb is the most significant (Table S6). The stepwise regression analysis was made between the lead content in root and shoot of *I. lactea* and the acid soluble Pb content in soil. The regression equation was as follows: $Y1= -222.58+18.99X$, $R^2=0.944$, $P<0.05$; $Y^2= -3.85+1.24X$, $R^2=0.810$, $P<0.05$, where $Y1$ and $Y2$ is the lead content (mg/kg) of shoot and root, respectively; X is the acid soluble lead content (mg/kg). The results confirmed that Pb absorbed by the root and shoot of *I. lactea* changed into acid soluble Pb in the soil. The addition of exogenous NO_3^--N had a positive impact on the form of Pb in the soil.

4.3 Effect of nitrate nitrogen addition on absorption and transport of lead

The low Pb content in the shoot of *I. lactea* will not stress the normal growth of *I. lactea*, but the amount of Pb it can absorb is far beyond the tolerance of most plants to Pb in nature (Yuan et al., 2015). Moreover, the addition of Pb did not affect the content and distribution proportion of various forms of Pb in the soil (Wang et al., 2018). Therefore, the direct effect of NO_3^--N may be the reason for the increase of Pb absorption in the root of *I. lactea*. It is presumed that the increase in NO_3^--N concentration brings more anion charge in the soil, prompting more anion uptake by the plant, which requires more cation uptake by the plant for balance, facilitating the membrane transport process of cations. Therefore, Pb content absorbed by the plant root increases. Moreover, the addition of exogenous NO_3^--N promotes the synthesis of organic acids in the soil. Organic acids may combine with Pb and assist in Pb translocation and accumulation (Monsant et al., 2010). Studies have proven that most of NO_3^--N absorbed with the aid of plant root desires to be reduced and utilized by plant, while a small part is stored in plant vacuoles as substances for ion balance and osmotic regulation. When NO_3^--N in the soil exceeds the upper limit of nitrogen required by

plants in growth period, it may cause ion imbalance in plants. Moreover, excessive nitrogen will also inhibit the absorption of other essential elements by plants, thus affecting the growth of plants (Kastl et al., 2015). In this experiment, under the condition of Pb_2 treatment, the addition of NO_3^--N substantially inhibited the normal growth of the aboveground part of *I. lactea*, but had a relatively small impact on the root. This is partly due to the stress effect of the rise of Pb concentration on the plant, but more is the inhibitory effect of the addition of exogenous NO_3^--N on the growth of *I. lactea*.

4.4 Relationship between nutrient absorption and transport of lead

Shoot N content of *I. lactea* negatively correlated with shoot P content, shoot and root Pb contents, and four forms of Pb (Fig. 6). Shoot N content of *I. lactea* positively correlated with root P content and shoot and root dry weights. There was a correlation between root N accumulation and shoot dry weight (Fig. 6). It can be noted that with the addition of NO_3^--N, the accumulation of P in root increased considerably, and most P element remained in plant root. N element is transported to the aboveground part of plant in huge quantities and contributes to the dry weight of plant (Xu et al., 2019). A small amount of N promotes plant dry weight and the absorption of Pb. With the increase of NO_3^--N concentration, the absorption capability of *I. lactea* to Pb decreases.

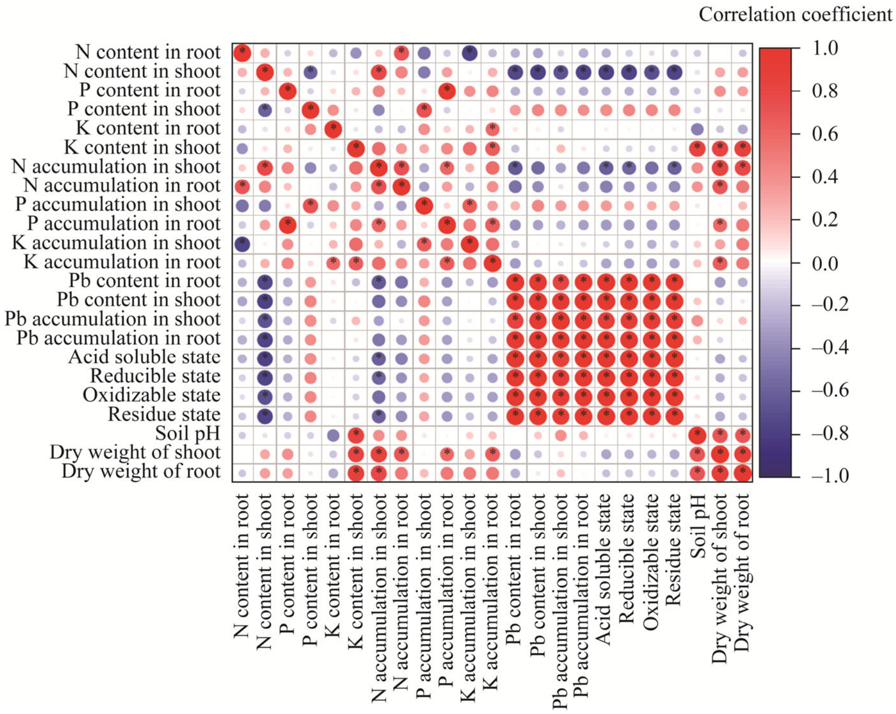

Fig. 6 Correlations of *Iris lactea* growth with absorption and transport of Pb. *, $P<0.05$ level.

5 Conclusions

The present study was conducted to study the effects of exogenous addition of NO_3^--N on the uptake and transport of Pb and nutrients, as well as the growth of *I. lactea*. The results found that exogenous NO_3^--N mainly affected the content of Pb in the root of *I. lactea*. Under different Pb concentrations, NO_3^--N application inhibited the transport of Pb from root to shoot. The higher the Pb concentration, the more significant the effect. This may be due to the balance effect of ions absorbed by the root of *I. lactea* caused by the addition of NO_3^--N. In the case of a certain degree of soil Pb pollution, the migration of heavy metals in plants can be improved by adjusting the level of soil nutrition. The addition of exogenous NO_3^--N reduces soil pH and affects the

distribution and content composition of different forms of Pb in the soil to a certain extent. This is due to the combination of NO_3^- and base ions leads to the reduction of soil pH underneath the motion of leaching. The demand for NO_3^--N could be very low in *I. lactea*. In the soil polluted by different concentrations of Pb, the addition of exogenous NO_3^--N can also promote the growth of *I. lactea*, but there is a limit in the concentration range of exogenous NO_3^--N. When the concentration of NO_3^--N is 0.1 g/kg, the promotion effect is the best.

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Appendix

Table S1 Effect of NO_3^--N on root/shoot ratio of *Iris lactea* in Pb-contaminated soil

NO_3^--N treatment	Pb_0	Pb_1	Pb_2
N_0	0.4988	0.5093	0.5202
N_1	0.5221	0.4553	0.5300
N_2	0.5485	0.4874	0.5584
N_3	0.5889	0.4887	0.5672

Note: N_0, 0.0 g/kg; N_1, 0.1 g/kg, N_2, 0.2 g/kg; N_3, 0.3 g/kg; Pb_0, 0 mg/kg; Pb_1, 800 mg/kg; Pb_2, 1600 mg/kg. The abbreviations are the same in the following tables.

Table S2 Effect of NO_3^--N on N, P, and K uptake and transportation of *Iris lactea* in Pb-contaminated soil

Nutrient element	Nitrate nitrogen treatment	Content in shoot (g/kg)			Content in root (g/kg)		
		Pb_0	Pb_1	Pb_2	Pb_0	Pb_1	Pb_2
N	N_0	20.82±1.52^{c}	20.49±1.36^{b}	15.60±1.34^{b}	9.57±1.02^{a}	10.46±0.33^{b}	8.60±1.33^{b}
	N_1	23.89±0.89^{a}	21.12±0.90^{b}	18.15±1.42^{a}	9.55±1.14^{a}	10.69±0.31^{b}	9.55±1.89^{a}
	N_2	22.77±0.33^{ab}	24.61±0.75^{a}	17.52±0.57^{ab}	10.55±1.02^{a}	13.70±0.20^{a}	9.96±1.00^{ab}
	N_3	22.12±1.47^{ab}	16.36±0.91^{c}	17.12±0.01^{ab}	11.24±0.51^{a}	13.54±3.06^{c}	10.17±0.70^{ab}
P	N_0	6.19±0.45^{a}	4.65±0.16^{b}	10.71±3.62^{ab}	10.28±1.16^{ab}	7.53±0.47^{a}	8.58±2.23^{a}
	N_1	6.27±0.29^{a}	6.72±0.69^{b}	8.79±0.26^{ab}	17.32±7.66^{a}	8.29±0.91^{a}	6.37±0.39^{a}
	N_2	6.19±0.07^{a}	6.81±1.02^{b}	13.47±3.43^{a}	2.82±0.38^{b}	8.69±0.68^{a}	6.73±0.48^{a}
	N_3	6.46±0.06^{a}	12.77±1.70^{a}	4.47±0.04^{b}	7.56±0.15^{b}	8.64±0.54^{a}	8.38±0.52^{a}
K	N_0	31.33±0.18^{a}	34.97±1.07^{a}	32.94±0.59^{a}	10.58±0.13^{b}	12.70±0.76^{ab}	12.48±1.37^{b}
	N_1	31.85±0.79^{a}	30.38±0.90^{b}	31.79±1.56^{a}	15.47±1.99^{a}	11.51±0.64^{b}	13.43±1.41^{ab}
	N_2	28.85±0.53^{b}	28.97±0.29^{c}	28.42±0.28^{b}	14.17±1.66^{a}	11.20±0.99^{b}	15.40±1.14^{a}
	N_3	28.46±1.06^{b}	28.61±0.40^{c}	24.88±1.06^{c}	13.00±0.16^{ab}	13.54±0.86^{a}	12.46±1.18^{b}

Note: Different lowercase letters within the same nutrient element and lead treatments indicate significant differences among different nitrate treatments at P<0.05 level. Mean±SD.

Table S3 Effect of NO_3^--N on N, P, and K accumulation of *Iris lactea* in Pb-contaminated soil

Nutrient element	Nitrate nitrogen treatment	Accumulation in shoot (mg/plant)			Accumulation in root (mg/plant)		
		Pb_0	Pb_1	Pb_2	Pb_0	Pb_1	Pb_2
N	N_0	1.50±0.06^{ab}	1.74±0.10^{a}	1.16±0.12^{a}	0.42±0.05^{a}	0.46±0.05^{a}	0.33±0.08^{a}
	N_1	1.94±0.17^{a}	1.84±0.12^{a}	1.15±0.15^{a}	0.40±0.05^{ab}	0.45±0.10^{b}	0.32±0.10^{a}
	N_2	1.40±0.03^{bc}	1.88±0.03^{a}	0.94±0.12^{ab}	0.36±0.04^{b}	0.48±0.01^{a}	0.30±0.04^{a}
	N_3	1.24±0.10^{c}	1.15±0.04^{b}	0.83±0.02^{b}	0.35±0.03^{b}	0.44±0.11^{b}	0.28±0.07^{a}
P	N_0	0.52±0.08^{a}	0.41±0.02^{c}	0.80±0.33^{ab}	0.37±0.04^{ab}	0.33±0.03^{a}	0.33±0.07^{a}
	N_1	0.52±0.01^{a}	0.60±0.01^{b}	0.52±0.10^{ab}	0.74±0.41^{a}	0.34±0.06^{a}	0.21±0.07^{ab}
	N_2	0.38±0.01^{b}	0.49±0.02^{c}	0.76±0.29^{a}	0.09±0.01^{b}	0.30±0.02^{a}	0.19±0.03^{b}
	N_3	0.36±0.03^{b}	0.88±0.06^{a}	0.22±0.00^{b}	0.24±0.02^{b}	0.30±0.05^{a}	0.22±0.04^{ab}
K	N_0	2.44±0.24^{a}	2.98±0.30^{a}	2.45±0.12^{a}	0.43±0.10^{b}	0.57±0.10^{a}	0.48±0.07^{a}
	N_1	2.58±0.24^{a}	2.64±0.11^{a}	2.01±0.37^{b}	0.64±0.00^{a}	0.48±0.12^{a}	0.45±0.10^{ab}
	N_2	1.78±0.02^{b}	2.12±0.16^{b}	1.52±0.19^{c}	0.49±0.10^{ab}	0.40±0.05^{a}	0.46±0.08^{ab}
	N_3	1.52±0.08^{b}	2.02±0.13^{b}	1.10±0.13^{c}	0.41±0.05^{b}	0.48±0.09^{a}	0.30±0.00^{b}

Note: Different lowercase letters within the same nutrient element and lead treatment indicate significant differences among different nitrate treatments at P<0.05 level. Mean±SD.

Table S4 Effect of NO_3^--N on transfer coefficients of N, P, and K of *Iris lactea* in Pb-contaminated soil

NO_3^--N treatment	N			P			K		
	Pb_0	Pb_1	Pb_2	Pb_0	Pb_1	Pb_2	Pb_0	Pb_1	Pb_2
N_0	2.18	1.96	1.81	0.60	0.62	1.25	2.96	2.75	2.64
N_1	2.50	1.98	1.90	0.36	0.81	1.38	2.06	2.64	2.38
N_2	2.16	1.80	1.76	2.20	0.78	2.00	2.05	2.59	1.85
N_3	1.97	1.21	1.68	0.85	1.48	0.53	2.19	2.11	2.00

Table S5 Effect of NO_3^--N on transfer coefficients of Pb of *Iris lactea* in Pb-contaminated soil

NO_3^--N treatment	Pb treatment	
	Pb_1	Pb_2
N_0	0.08	0.10
N_1	0.06	0.08
N_2	0.08	0.06
N_3	0.08	0.05

Table S6 Correlation between Pb forms in soil and contents of Pb in shoot and root of *Iris lactea*

Index	Acid soluble state	Reducible state	Oxidizable state	Residual state
Pb content in root	0.972^{**}	0.946^{**}	0.889^{**}	0.897^{**}
Pb content in shoot	0.900^{**}	0.877^{**}	0.859^{**}	0.875^{**}

Note: **, $P<0.01$ level.